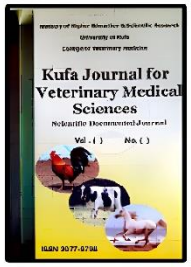
Kufa journal for Veterinary Medical Sciences Vol.(15) No.(1)

Kufa journal for Veterinary Medical Sciences

Kj.vs@uokufa .edu.iq

www.uokufa .edu.iq\journals\index.php\kjvs



Hematoprotective Effect of Eugenol against Iron Overload in Male Rats

Ahmed M. Malik Al-Mashhadi[1] **Nabeel Mohammed Al-Sharafi**[1]

[1]Department of Physiology, Biochemistry, and Pharmacology, Faculty of Veterinary Medicine University of Kufa, Iraq

Corresponding author: AMA, e-mail: ahmedm.almashehdi@uokufa.edu.iq
Co-author: NMA, e-mail: nabeelm.naji@uokufa.edu.iq

Received date: 29 February, 2024 **Accepted:** 10 June, 2024 **page:** (20-33) **Published:** 30 June, 2024

DOI: https://doi.org/10.36326/kjvs/2024/v15i115324

Abstract:

This study aimed to evaluate the hematoprotective effects of eugenol against iron overload in male rats. A total of 30 rats were randomly divided into six equal groups as follows: the control negative (C-) group I.P injection distal water. The control positive (C+) group received iron dextran (100 mg/kg-BW) I.P injection. The IE1 and IE2 groups received iron dextran 100 mg/kg-BW I.P and eugenol (50,100 mg/kg-BW) orally, respectively, and the E3 and E4 groups received eugenol (50,100 mg/kg-BW) orally. After 30 days of the experiment, the blood was collected to measure the experimental parameters. The results demonstrated a significant increase in RBCs, Hb percentage, WBCs, PCV, and PTL in the C+ group compared to the C- group. Also, there was a non-significant difference between IE1 and C+ in RBCs and Hb. Meanwhile, PCV decreased significantly in IE1 compared with C+. Conversely, the C+ group showed decreased MCV compared with IE1. Microscopic examination of blood smears indicated normal red blood cell morphology in the C- and iron dextran with eugenol-treated groups, while the C+ group exhibited microcytic hypochromic morphology. In conclusion, the ameliorative effect of eugenol against iron overload-induced hematotoxicity is achieved by restoring the hematological parameters near the normal control.

Keywords: Blood smear staining, Complete blood count, Eugenol, Hematotoxicity, Iron overload.

Introduction:

The hematopoietic system is responsible for the persistent produces of extremely specialized blood cells in the bodies [1]. Hematotoxicity is described as the retrograde sequence of a laborer on blood or blood-forming organs [2, 3].

Iron overload refers to the accumulation of excessive iron deposits or labile forms of iron within almost every part of the body, which is potentially harmful and causes continuous toxicity damage to a variety of organ diseases, such as those that affect the kidneys, heart, and liver [4, 5]. The accumulation of excess iron in the specific individuals can be linked to various sources, including genetic predispositions such as hereditary hemo-chromatosis, repeated transfusions of red

blood cells, and using iron treatment by injections in cases of anemia that necessitate transfusions [6]. Previous studies have substantiated the association between ferroptosis and the toxic effects of iron overload in the kidney. The mechanisms underlying ferroptosis brought on by metals encompass mitochondrial stress, which produces an excessive amount of superoxide anion. This, in turn, inhibits the Nrf2-HO-1/GPX4 antioxidant system, amplifying the release of labile iron and consequently fostering ferroptosis, oxidative stress, lipid peroxidation, and ferritinophagy [7, 8-9].

Eugenol (EUG) is a phenolic chemical compound widely used in traditional medicine and is a versatile essential oil from various plants, including clove extract. The impact of its consumption on healthy individuals' liver and kidney morphology and physiology remains uncertain, with no clear determination of whether it induces positive or negative effects [10, 11]. EUG is a critically important component in various food products found in soybeans, clove oil, camphorated oil, basil, nutmeg, cinnamon, and bay leaf [12]. Eugenol (EUG) exhibits diverse health-promoting bioactivities, including antioxidant, anti-inflammatory, antiseptic, and analgesic properties, making it a potential therapeutic agent for chronic kidney diseases [13]. Eugenol also demonstrates protective effects on the gastrointestinal tract and possesses antimicrobial [14], antifungal [15], and antiviral properties [16]. Recent studies explain that the protective role of eugenol safeguards against drug-induced kidney injury [17, 18]. Moreover, eugenol's possible modulation effect on biochemical and histological Triggered by Silver Nanoparticles induced nephrotoxicity [19].

Thus, our study aimed to investigate the protective roles of Eugenol against the hematotoxicity induced by iron overload in male rats.

Materials and Methods:

Animal Ethical Statement:

This study was approved by the Scientific Committee of the Faculty of Veterinary Medicine, University of Kufa, and confirms to the ethical principles of care and Laboratory animals (reference number UK.VET.2023/11/7.27151).

Experimental animals:

Thirty male albino rats weighing (200 – 250 g). The rats were kept in the animal house at the Faculty of Veterinary Medicine University of Kufa. They were placed in clean plastic cages, with five rats per cage, and wood was shared for bedding. The animals were kept in a controlling room temperature between (23 and 25 °C), with a 12-hour light/dark cycle and pleasing environment ventilation. The animals were maintained for around two weeks for adaptation before the experiment started. During the experiments, they had unrestricted access to regular pellets and water (*ad libitum*).

Experiment design:

The animals were randomly divided into six equal groups (n=5 for each) of treatment administrations during 30 experimental days. Control Negative Group (C-): Five healthy male rats were injected I.P. with distal water. Control Positive Group(C+): Five male rats received iron dextran (LYFEXT ®/USP/ India BN: ML22395) 100 mg/kg-BW I.P. injection every 72 hours. Group IE1**:** Five male rats received iron dextran 100 mg/kg-BW I.P. injection every 72 hours and orally gavages eugenol (Solarbio-China CN: 97-53-0) 50 mg/kg -BW per day. Group IE2**:** Five male rats received an iron dextran 100 mg/kg-BW I.P. injection every 72 hours and were given eugenol 100 mg/kg -BW daily. Group E3: Five male rats received orally eugenol 50 mg/kg -BW per day. Group E4**:** Five male rats received 100 mg/kg -BW of eugenol orally per day. After the end of the experiment, the animals were prepared for anesthetizing using intramuscular injection of (Ketamine 80 mg/kg B.W.) combined with (Xylazine 8 mg /kg B.W.) using a sterile syringe [20]. Blood samples were collected via cardiac puncture technique for measurement CBC count (Hematology Analyzer - Count Vet) using a device (GENEX -USA)) and blood smear staining.

Statistical Analysis:

The experimental data were statistically analyzed using Graph Pad Prism version 7. A one-way analysis of variance (ANOVA) was conducted to assess the significance of differences between groups, followed by Tukey multiple comparisons. The data was presented as mean ± standard errors of the mean (SEM), and statistical significance was determined by a P value<0.05 [21].

Results:

The effects of iron dextran injection and oral eugenol administration on hematological parameters in male rats with hematotoxicity caused by iron overload:

A- Complete Blood Counts (CBC)

1- Red Blood Cells Count

Figure (1) depicts the influence of iron dextran injection and daily oral eugenol administration on red blood cell (RBC) count cell/L in male albino rats. The red blood cell (RBC) count showed a statistically significant increase (P ≥ 0.05) in the C+ group compared to the C- group. However, no significant difference was seen between the other groups (IE1, IE2, E3, and E4). Notably, the RBC count showed no significant difference in the IE2 group compared to the eugenol-treated groups (E3 and E4), whereas it exhibited a significant increase in the IE1 group.

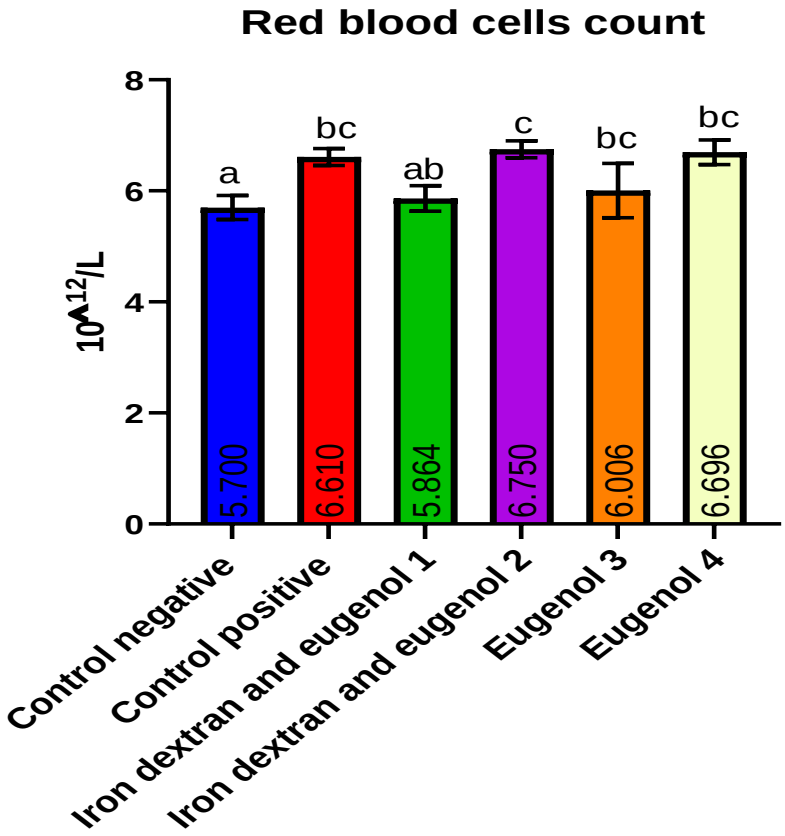


Figure 1: Effect of eugenol orally and iron dextran injection on red blood cell (RBC) count cell/L in male albino rats. The different letters explained the significant differences among groups, while the similar letters denote non-significant differences among groups. The error bars explain mean ± SEM for p-value at (p ≥ 0.05); ANOVA one-way statistical analysis was used to calculate the significance differences among groups.

2- White Blood Cells Count

Figure (2) illustrates the effects of iron dextran injection and daily oral eugenol administration on white blood cell (WBC) count cell/L in male albino rats. The WBC count exhibited a significant increase (P ≥ 0.05) in the C+ group compared with all experimental groups. In contrast, the eugenol-treated groups showed a non-significant

difference observed ($P \leq 0.05$) with the C- group when compared with each other.

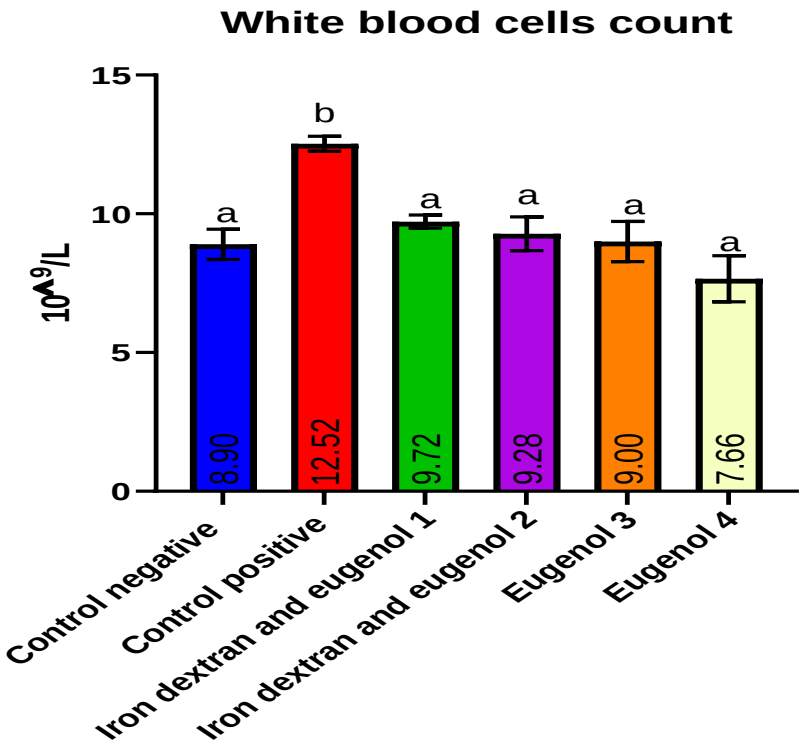


Figure 2: Effect of eugenol orally and iron dextran injection on white blood cell (WBC) count cell/L in male albino rats. The different letters explained the significant differences among groups, while the similar letters denote non-significant differences among groups. The error bars explain mean ± SEM for p-value at ($p \geq 0.05$); ANOVA one-way statistical analysis was used to calculate the significance differences among groups.

3- Hemoglobin Level Evaluation

Figure (3) depicts the influence of iron dextran injection and daily oral eugenol administration on Hemoglobin Level (Hb) g/dL in male albino rats. The Hb demonstrated a significant increase ($P \geq 0.05$) in the C+ group compared with the C- group, while a non-significant difference was observed with the other groups (IE1, IE2, E3, and E4). Notably, the Hb showed a non-significant difference in the IE1 group compared to the eugenol-treated groups (IE2, E3, and E4). Additionally, the C- group displayed a significantly lower Hb than all other groups except for E3.

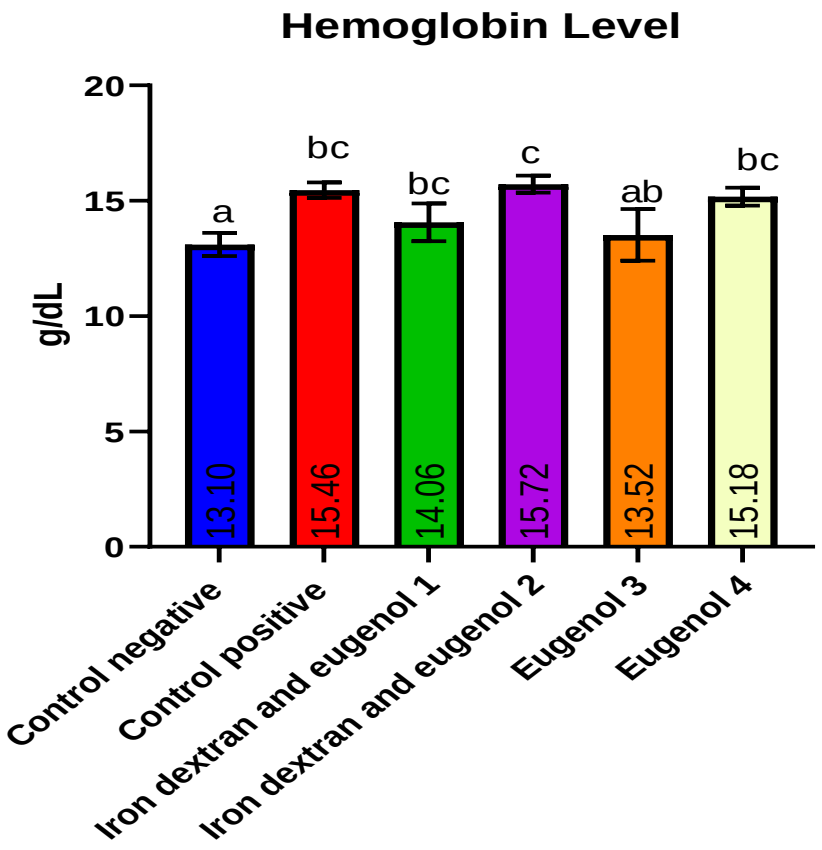


Figure 3: Effect of eugenol orally and iron dextran injection on hemoglobin Level (Hb) g/dL in male albino rats. The different letters explained the significant differences among groups, while the similar letters denote non-significant differences among groups. The error bars explain mean ± SEM for p-value at ($p \geq 0.05$); ANOVA one-way statistical analysis was used to calculate the significance differences among groups.

4- Packed Cell Volume percentage

Figure (4) illustrates the effect of iron and different concentrations of eugenol on Packed Cell Volume (PCV) percentage after 30 days of treatment. The control positive C+ group exhibited a significant increase in PCV % compared to all other groups ($P \geq 0.05$). Interestingly, among the eugenol-treated groups, there were non-significant differences in PCV % when compared to each different IE1, IE2, and E4 groups, while E3 decreased significantly among all eugenol groups. Also, non-significantly were compared with the C- group.

5- Means Corpuscular Volume

Figure (5) illustrates the effect of iron and different concentrations of eugenol on Means Corpuscular Volume (MCV) fL after 30 days of treatment. The control positive C+ group showed a significant decrease in MCV fL compared with all treated groups ($P \leq 0.05$). Also, non-significant ($P \geq 0.05$) between C- group compared with IE1, IE2, E3, and E4 groups.

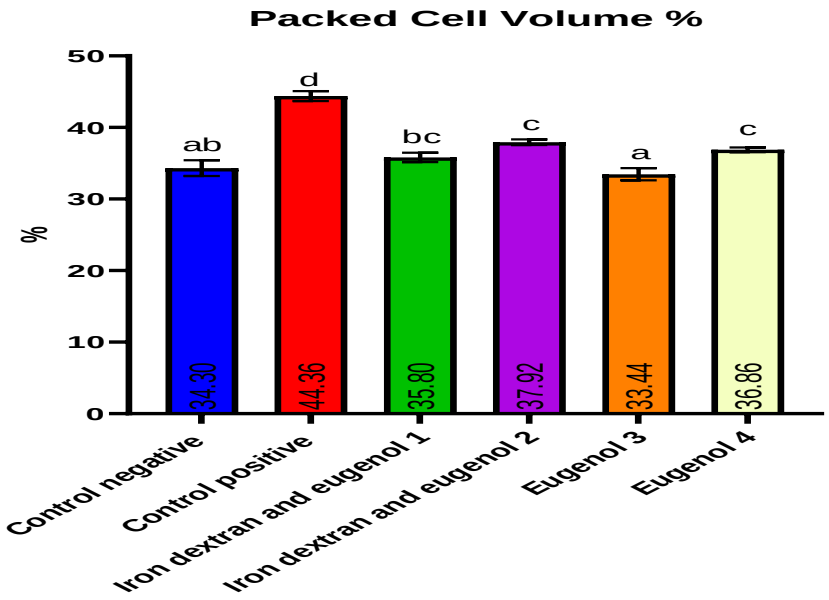


Figure 4: Effect of eugenol orally and iron dextran injection on Packed Cell Volume percentage % in male albino rats. The different letters explained the significant differences among groups, while the similar letters denote non-significant differences among groups. The error bars explain mean ± SEM for p-value at ($p \geq 0.05$); ANOVA one-way statistical analysis was used to calculate the significance differences among groups.

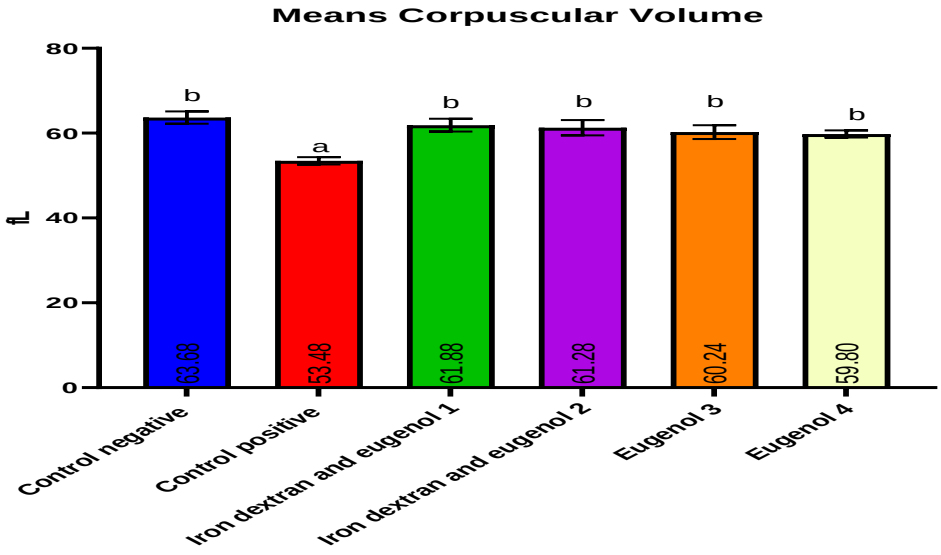


Figure 5: Effect of eugenol orally and iron dextran injection on means corpuscular volume (MCV) fL in male albino rats. The different letters explained the significant differences among groups, while the similar letters denote non-significant differences among groups. The error bars explain mean ± SEM for p-value at ($p \geq 0.05$); ANOVA one-way statistical analysis was used to calculate the significance differences among groups.

6- Platelet Cells Counts

Figure (6) illustrates the effect of iron and different concentrations of eugenol on Platelet Cells (PLT) cell/L after 30 days of treatment. The control positive C+ group exhibited a non-significant difference compared to IE1 and IE2 groups which increased significantly ($P \leq 0.05$). Interestingly, the result of the IE1 group showed no significance compared with (C- and IE2). On the other hand C- group

showed non-significantly compared with eugenol alone (E3 and E4) groups.

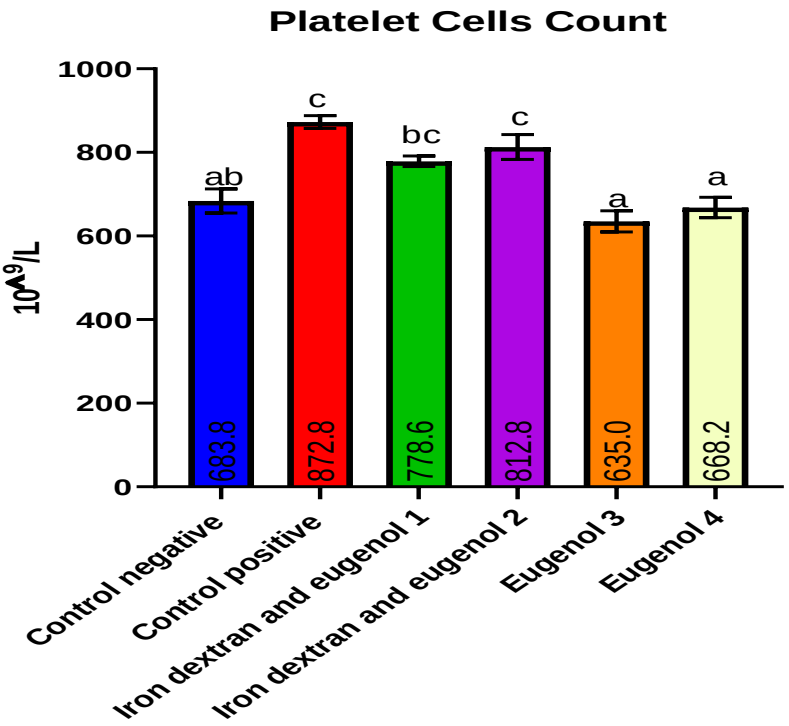


Figure (6): Effect of eugenol orally and iron dextran injection on Platelet Cells (PLT) cell/L in male albino rats. The different letters explained the significant differences among groups, while the similar letters denote non-significant differences among groups. The error bars explain mean ± SEM for p-value at ($p \geq 0.05$); ANOVA one-way statistical analysis was used to calculate the significance differences among groups.

B- Blood Smear Staining Process:

The microscopic examination of the control negative groups (C-) showed no changes in the morphology of red blood cells in the figures (7 A). Also, with eugenol 50, eugenol 100, iron dextran and eugenol 50, and iron dextran and eugenol 100 (E3, E4, IE1, IE2) treated groups, showed normal red blood cell morphology in the figures (8 A, B, C, and D).The blood smear results in control positive groups (C+) iron dextran injection showed changes in reddest blood cells' microcytic hypochromic morphology appearance smaller and paler red blood cells in figures (7 B).

Discussion:

The present study was conducted to investigate the protective effects of eugenol against iron-induced hematotoxicity in male rats to explore hematological and blood smear parameters to assess eugenol's impact on renal function. The results of the present study showed that a complete blood count examination in male albino rats subjected to iron dextran injection and daily oral eugenol administration revealed significant variations in hematological parameters. The result of the C- group in the everyday physiological context shows that the red blood cells (RBCs) are in a normal range with hemoglobin (Hb) concentration. However, in the context of the iron overload C+ group, where there is an excess of iron in the body, the relationship between RBC and iron is more complex since the iron overload leads to oxidative stress agent damage in various tissues, including the kidneys, these agents can involve free superoxide radicals and hydrogen peroxide [22, 23-24].

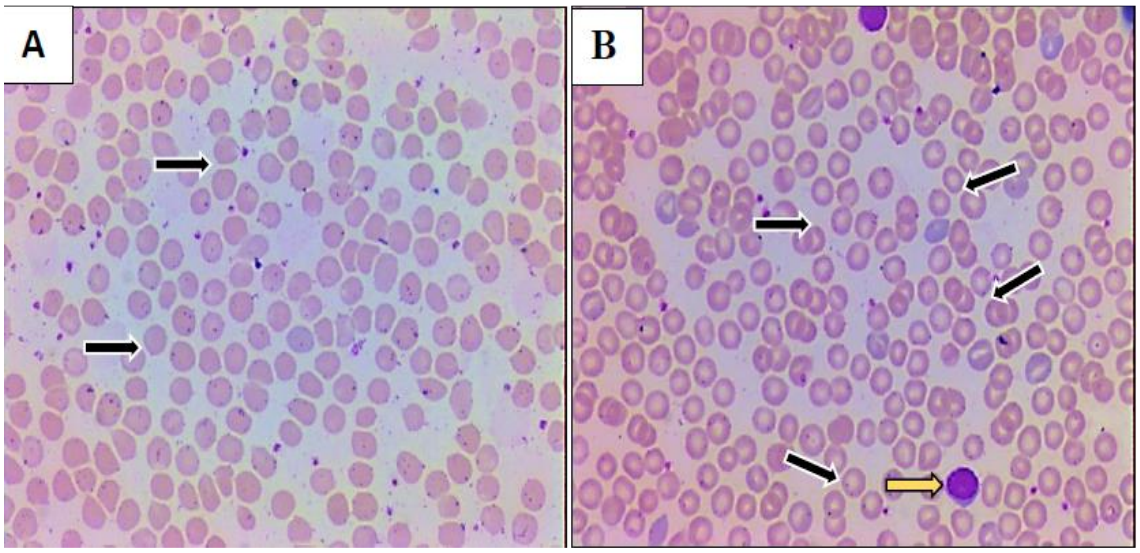


Figure (7- A and B): Photomicrograph of control negative and control positive groups effect of iron dextran injection on blood smears.
A/ Control negative group. Normal red blood cells morphology (black arrow). **B/** Control positive group iron dextran injection. Most red blood cells showed abnormal microcytic hypochromic morphology, more minor and paler red blood cells. Note lymphoblast (yellow arrow). **Wright stain. A and B: 400x.**

Elevated iron levels trigger a physiological response involving the release of erythropoietin (EPO) from the kidneys, particularly in the kidneys, and to support the continued production of RBCs and hemoglobin, there can be an increase in erythropoietin production as a compensatory mechanism since the increase in red blood cells (RBC) and hemoglobin (Hb) levels during iron overload due to the role of iron in erythropoiesis and hemoglobin synthesis [25, 26]. Iron overload may be an attempt by the body to counteract the negative impact of iron-induced tissue damage. EPO, a hormone, stimulates the bone marrow to intensify the production of red blood cells. Simultaneously, the excess iron serves as a critical component for hemoglobin formation, with each hemoglobin molecule containing four iron atoms. This abundance of iron optimizes the efficiency of hemoglobin synthesis, contributing to an elevated hemoglobin concentration within the red blood cells. This process initially promotes increased RBC and Hb levels [26]. On the other hand, the RBC and Hb levels exhibited a significant reduction in the iron-treated groups IE1 and IE2 compared to the control positive C+ group, indicating a potential impact of iron overload on erythropoiesis; interestingly, the E4 group, receiving high-dose eugenol, demonstrated the highest RBC and Hb levels among all groups. Suggesting a modulatory effect of eugenol on erythropoietin regulation, those investigating the hematopoietic and antioxidant effects of eugenol are essential to understand further the potential therapeutic implications of eugenol in iron-induced hematological alterations [27, 28-29]. The alterations observed in the white blood cell (WBC) count following iron dextran injection and daily oral eugenol administration in male albino rats enhance immune effects and organ-specific inflammation [30].

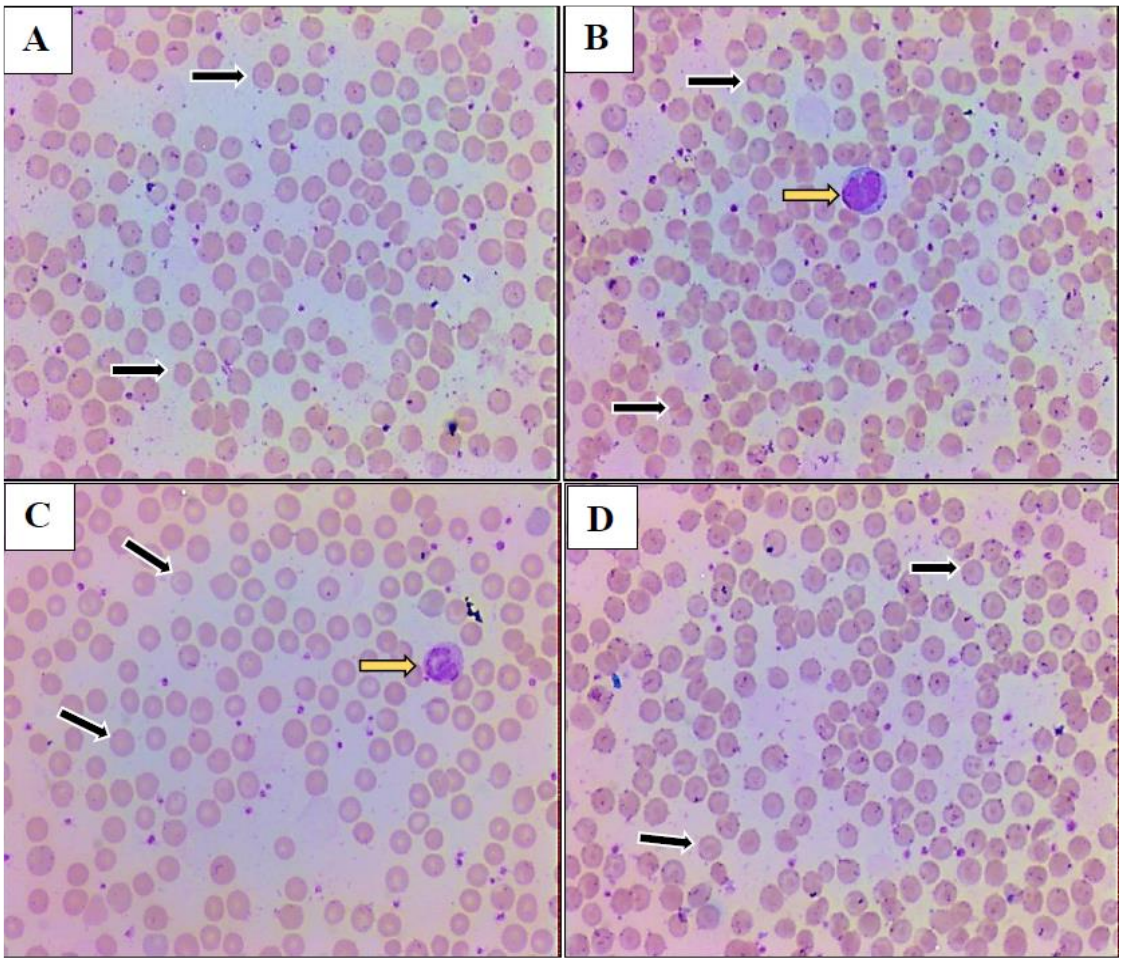


Figure (8 A, B, C, and D): Photomicrograph effect of eugenol orally and iron dextran injection on blood smears.
A/ Eugenol 50 mg/kg B.W (E3) treated group**.** Normal red blood cell morphology (black arrow**).** B/ Eugenol 100 mg/kg B.W (E4) treated group**.** Normal red blood cell morphology (black arrow)**.** Note lymphoblast (yellow arrow)**.** C/ Iron dextran and eugenol 50 mg/kg B.W (IE1) treated group**.** Normal red blood cell morphology (black arrow). Note neutrophil (yellow arrow). D/ Iron dextran and eugenol 100 mg/kg B.W (IE2) treated group**.** Normal red blood cell morphology (black arrow)**.** Wright stain. A, B, C, and D: 400x.

In a typical inflammation, the lymphocytes, encompassing B cells and T cells, contribute to antibody production and the identification and elimination of infection-causing cells, showing the pivotal role of WBCs in immune responses against inflammation [31]. In response to infections or inflammatory conditions, the body releases WBCs to decrease harmful. [32, 13]. The current results indicate that the white blood cell (WBC) count increased in the C+ group compared to the IE1 and C-groups due to iron overload, which led to extra-medullary hematopoiesis [33]. Iron overload triggers the production of reactive oxygen species (ROS). Unregulated buildup of excessive iron can result in oxidative stress, damaging cellular components [34]. The mechanism by which iron overload affects WBCs involves a cascade of events instigated by the iron's engagement in the Fenton reaction, generating ROS and free radicals. Particularly in its Fe2+ state, excessive iron promotes redox cycling, catalyzing the production of hydroxyl radicals upon interaction with molecular oxygen. These highly reactive radicals, in turn, invade cell membranes, causing DNA damage [35]. Interestingly, the lE2 group exhibited a significant increase in WBC count compared to the 1E1, E3, and E4 group's findings suggest that iron dextran injection and eugenol administration exert influences on

WBC count, indicating potential repercussions for immune response and overall health in the experimental rat model. This results in the effects of eugenol on the reduction of WBC compared with the control group, depending on the doses of eugenol, which has anti-inflammatory effects by modulating cytokine production and inhibiting inflammatory pathways [17]. The observed variations in WBC count are attributed to possible immunosuppressive effects, anti-inflammation effects of eugenol inhibition of the WBC, oxidative stress, impacts on hematopoiesis, and the intricate interplay of responses induced by iron overload [36, 13].

The alteration of packed cell volume (PCV %) in male albino rats following a 30-day treatment with iron dextran and various doses of eugenol shows diverse patterns. The C+ group showed a substantial rise in PCV % compared to the other groups, suggesting that the injection of iron dextran resulted in a large increase in PCV [37]. This result is consistent with the established function of iron in hematopoiesis, where elevated iron levels can promote the synthesis of red blood cells and impact PCV. Notably, there were no statistically significant variations in PCV% among the eugenol-treated groups (IE1, IE2, E3, and E4) compared to the C-group. This indicates that oral eugenol concentrations of varying strengths reduced PCV% to the range observed in the control group [38]. Eugenol, recognized for its anti-inflammatory effects, has impacted PCV by reducing inflammation and oxidative stress, leading to a reduction observed in the E3 group [39].

The observed impact on Mean Corpuscular Volume (MCV) fL indicates that iron dextran injection strongly influences MCV, leading to a notable decrease caused by iron overload, a major component affecting changes in RBC properties [40, 41]. Excess iron levels in the body, associated with oxidative stress and disruption of erythropoiesis, contribute to the observed alteration in mean corpuscular volume [42]. On the other hand, the no significant differences between the control negative group (C-) and the groups treated with eugenol (IE1, IE2, E3, and E4) indicates that eugenol, whether supplied alone or in administration with iron dextran, does not have a significant impact on MCV compared to the control groups [43]. This leads to the potential protective effect of eugenol, which can be cause attributed to its antioxidant properties [43], mitigating the oxidative stress induced by iron overload [44]. The observed changes in MCV since the intricate interplay between iron status, oxidative stress, and erythropoietic processes in RBC development [45] .

The Platelet cell count (PLT) findings suggest that iron dextran injection significantly impacts Platelet Cell count, which increased significantly. Furthermore, iron overload affects platelet function due to its role in hematopoiesis. Studies have proved that iron overload through the increased levels of ROS increases platelets, which play a crucial role in platelet receptor activation [46]. Additionally, it impacts platelet function by affecting other organs, such as the liver and kidney, in which iron is involved in multiple intracellular and extracellular processes, influencing the course of clonal myeloid disorders of PLT formation [47]. The result showed that eugenol caused a decrease in the platelet to average level compared to control in all groups (E1, IE2, E3, and E4) due to the antioxidant effects of the eugenol as described previously [32,13].

The observed effect of iron overload and eugenol on blood film smear morphology involves processes associated with erythropoiesis, iron homeostasis, and oxidative stress. Blood smear in the control negative (C-) group showed the appearance of typical normal RBCs in the shape, size, and stain compared with the control positive (C+) group iron overload, induced by iron dextran injection, demonstrated significant changes in RBC morphology, disrupts normal hemoglobin synthesis and erythrocyte maturation, leading to the production of microcytic and hypochromic red blood cells (RBCs) and characterized smaller, paler RBCs. The excess iron precipitates leading to increased oxidative stress in RBCs and deformability of RBC membrane, reactive oxygen species (ROS) that cause damage to cellular components have been demonstrated in previous reports [48,49]. Groups treated

with eugenol at different doses (IE1, IE2, E3, and E4) and combined with iron dextran injection exhibit normal RBC morphology. Eugenol, recognized for its antioxidant properties, intervenes in this process by scavenging free radicals and mitigating oxidative damage through counteracting oxidative stress [50]. Eugenol protects against the adverse effects of iron overload, preserving the typical morphology of RBCs in the blood smear [36]. This suggests a multifaceted mechanism involving modulation of iron metabolism, amelioration of oxidative stress, and preservation of erythropoietic integrity, which is the potential of eugenol as a therapeutic agent against iron-induced hematological changes in blood smear morphology [29].

Conclusions:

In conclusion, eugenol's ameliorative effect against iron overload-induced hematotoxicity is manifested by restoring the hematological parameters and blood smear section near the standard control.

Conflict of Interest:

There is no conflict of interest in the publication of this paper.

Acknowledgment:

We want to express our gratitude and recognition to the Department of Physiology, Biochemistry, and Pharmacology within the Faculty of Veterinary Medicine at the University of Kufa for providing the necessary facilities to conduct this research.

References:

1. Ramaiah L, Bounous DI, Elmore SA. Hematopoietic system. InHaschek and Rousseaux's handbook of Toxicologic pathology. 3rd ed. Academic Press; 2013, pp. 1863-1933. doi.org/10.1016/B978-0-12-415759-0.00050-9.

2. Akhter MS, Rashid H, Hamali HA, Alshahrani S, Dobie G, Qadri M, Madkhali AM, Mobarki AA. Hematotoxicity of Co-Administration of Bisphenol A and Acetaminophen in Rats and its Amelioration by Melatonin. Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders). 2023; 232:222-229. doi.org/10.2174/1871530322666220607124757.

3. Budinsky Jr RA. Hematotoxicity: chemically induced toxicity of the blood. Principles of Toxicology: 2nd ed; In: Phillip LW., Robert CJ, Stephen MR, Editors. Environmental and Industrial Applications: E-Publishing Inc; 2000, 17:87-109. doi.org/10.1002/0471231800.ch4.

4. Fernandez M, Lokan J, Leung C, Grigg A. A critical evaluation of the role of iron overload in fatty liver disease. Journal of gastroenterology and hepatology. 2022; 37.10:1873-83. doi.org/ 10.1111/jgh.15971.

5. Srivastava NK, Mukherjee S, Mishra VN. One advantageous reflection of iron metabolism in context of normal physiology and pathological phases. Clinical Nutrition ESPEN. 2023; 58.14: 277-294. doi.org/ 10.1016/j.clnesp.2023.10.006.

6. Heriatmo NL, Estuningtyas A, Soetikno V. Iron-Overload Conditions: Manifestations to the Kidney Organs–A Review. Borneo Journal of Pharmacy. 2023; 6.4:360-369. doi.org/10.33084/bjop.v6i4.4411.

7. Qiu Y, Cao Y, Cao W, Jia Y, Lu N. The application of ferroptosis in diseases. Pharmacological Research. 2020; 1.159:104919. doi.org/ 10.1016/j.phrs.2020.104919.

8. Song X, Long D. Nrf2 and ferroptosis: a new research direction for neurodegenerative diseases. Frontiers in neuroscience. 2020; 14:267. doi.org/ 10.3389/fnins.2020.00267.

9. Aschner M, Skalny AV, Martins AC, Sinitskii AI, Farina M, Lu R, Barbosa Jr F, Gluhcheva YG, Santamaria A, Tinkov AA. Ferroptosis as a mechanism of non-ferrous metal toxicity. Archives of Toxicology. 2022; 96.9:2391-417. doi.org/ 10.1007/s00204-022-03317-y.

10. Fu X, Gao Y, Yan W, Zhang Z, Sarker S, Yin Y, Liu Q, Feng J, Chen J. Preparation of eugenol nanoemulsions for antibacterial activities. RSC advances. 2022; 12.6:3180-90. doi.org/10.1039/d1ra08184e.

11. Carvalho RP, Ribeiro FC, Lima TI, Ervilha LO, de Oliveira EL, de Oliveira Faustino A, de Almeida Lima GD, Machado-Neves M. High doses of eugenol cause structural and functional damage to the rat liver. Life Sciences. 2022; 1.304:120696. doi.org/10.1016/j.lfs.2022.120696.

12. Hossain MB, Ahmed L, Martin-Diana AB, Brunton NP, Barry-Ryan C. Individual and Combined Antioxidant Activity of Spices and Spice Phenolics. Antioxidants. 2023; 12.2:308. doi.org/10.3390/antiox12020308.

13. Fathy M, Abdel-Latif R, Abdelgwad YM, Othman OA, Abdel-Razik AR, Dandekar T, Othman EM. Nephroprotective potential of eugenol in a rat experimental model of chronic kidney injury; targeting NOX, TGF-β, and Akt signaling. Life Sciences. 2022; 1.308:120957. doi.org/10.1016/j.lfs.2022.120957.

14. Mak KK, Kamal M, Ayuba S, Sakirolla R, Kang YB, Mohandas K, Balijepalli M, Ahmad S, Pichika M. A comprehensive review on eugenol's antimicrobial properties and industry applications: A transformation from ethnomedicine to industry. Pharmacognosy Reviews. 2019; 13.25:1-9. Doi.org/10.4103/phrev.phrev_46_18.

15. Ibrahim OM, Ali BM. Antifungal potency of clove (Syzygium aromaticum) essential oil extract against induced systemic infection by Candida albicans in mice. Kufa Journal For Veterinary Medical Sciences. 2023; 14.1:17-26. doi.org/10.36326/kjvs/2023/v14i111158.

16. El-Saber Batiha G, Magdy Beshbishy A, El-Mleeh A, M. Abdel-Daim M, Prasad Devkota H. Traditional uses, bioactive chemical constituents, and pharmacological and toxicological activities of Glycyrrhiza glabra L.(Fabaceae). Biomolecules. 2020; 10.3:352. doi.org/ 10.3390/biom10030352.

17. Barhoma RA. The role of eugenol in the prevention of chromium-induced acute kidney injury in male albino rats. Alexandria journal of medicine. 2018; 54.4:711-715. doi.org/10.1016/j.ajme.2018.05.006.

18. Kadir A, Sher S, Siddiqui RA, Mirza T. Nephroprotective role of eugenol against cisplatin-induced acute kidney injury in mice. Pakistan Journal of Pharmaceutical Sciences. 2020; 33.3:1281-1287. doi.org/10.36721/PJPS.2020.33.3.SUP.1281-1287.1.

19. Aboelwafa HR, Ramadan RA, Ibraheim SS, Yousef HN. Modulation Effects of Eugenol on Nephrotoxicity Triggered by Silver Nanoparticles in Adult Rats. Biology. 2022; 11.12:1719. doi.org/10.3390/biology11121719.

20. Jirkof P, Lofgren J. Anesthesia and analgesia in laboratory rodents. In Anesthesia and Analgesia in Laboratory Animals. 3rd ed. Academic Press; American College of Laboratory Animal Medicine; 2023, pp. 287-356.doi.org/10.1016/B978-0-12-822215-7.00007-X.

21. Ntumi S. Reporting and interpreting One-Way Analysis of Variance (ANOVA) using a data-driven example: A practical guide for social science researchers. Journal of Research in Educational Sciences (JRES). 2021; 12.14:38-47. doi.org/10.14505/jres.v12.14.04.

22. Galaris D, Pantopoulos K. Oxidative stress and iron homeostasis: mechanistic and health aspects. Critical reviews in clinical laboratory sciences. 2008; 45.1:1-23. doi.org/10.1080/10408360701713104.

23. Yun S, Chu D, He X, Zhang W, Feng C. Protective effects of grape seed proanthocyanidins against iron overload-induced renal oxidative damage in rats. Journal of Trace Elements in Medicine and Biology. 2020; 57:126407. doi.org/10.1016/j.jtemb.2019.126407.

24. Mohammad G, Matakidou A, Robbins PA, Lakhal-Littleton S. The kidney hepcidin/ferroportin axis controls iron reabsorption and determines the magnitude of kidney and systemic iron overload. Kidney international. 2021; 100.3:559-69. doi.org/10.1016/j.kint.2021.04.034.

25. Dang J, Jia R, Tu Y, Xiao S, Ding G. Erythropoietin prevents reactive oxygen species generation and renal tubular cell apoptosis at high glucose level. Biomedicine

& Pharmacotherapy. 2010; 64.10:681-5. doi.org /10.1016/j.biopha.2010.06.011.

26. Sun Y, Liu G, Jiang Y, Wang H, Xiao H, Guan G. Erythropoietin Protects Erythrocytes Against Oxidative Stress-Induced Eryptosis In Vitro. Clinical laboratory. 2018; 64.3:365. doi.org/10.7754/Clin.Lab.2017.170924.

27. Arab HH, Salama SA, Maghrabi IA. Camel milk ameliorates 5-fluorouracil-induced renal injury in rats: targeting MAPKs, NF-κB and PI3K/Akt/eNOS pathways. Cellular Physiology and Biochemistry. 2018; 46.4:1628-42. doi.org/10.1159/000489210.

28. Mateen S, Rehman MT, Shahzad S, Naeem SS, Faizy AF, Khan AQ, Khan MS, Husain FM, Moin S. Anti-oxidant and anti-inflammatory effects of cinnamaldehyde and eugenol on mononuclear cells of rheumatoid arthritis patients. European journal of pharmacology. 2019; 852:14-24. doi.org/ 10.1016/j.ejphar.2019.02.031.

29. Salim YM, EL-Gendy IR, Nassae AM. Toxicological effects of methyl eugenol and fenitrothion on hematological, hepatic, renal, and oxidative stress-related biochemical characteristics of white albino rats. Interdiscip Toxicol. 2020; 13:13-20. doi.org/ 10.2478/intox-2020-0002.

30. Wang D, Wang S, Zhou Z, Bai D, Zhang Q, Ai X, Gao W, Zhang L. White blood cell membrane-coated nanoparticles: recent development and medical applications. Advanced Healthcare Materials. 2022; 11.7:2101349.
doi.org/10.1002/adhm.202101349

31. Fathabad SG, Kurzhagen JT, Sadasivam M, Noel S, Bush E, Hamad AR, Rabb H. T lymphocytes in acute kidney injury and repair. In Seminars in Nephrology; WB Saunders: 2020; 40.2:114-125. doi.org/10.1016/j.semnephrol.2020.01.003.

32. Said MM. The protective effect of eugenol against gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. Fundamental & clinical pharmacology. 2011; 25.6:708-16. doi.org/10.1111/j.1472-8206.2010.00900.x.

33. Sadek SA, Marzouk M, Mohamed HR, El-sallam BF, Elfiky AA, Sayed AA. Chia seeds and coenzyme Q10 alleviate iron overload induced hepatorenal toxicity in mice via iron chelation and oxidative stress modulation. Scientific Reports. 2023; 13.1:19773. doi.org/ 10.1038/s41598-023-47127-3.

34. Chai X, Li D, Cao X, Zhang Y, Mu J, Lu W, Xiao X, Li C, Meng J, Chen J, Li Q. ROS-mediated iron overload injures the hematopoiesis of bone marrow by damaging hematopoietic stem/progenitor cells in mice. Scientific Reports. 2015 May 13; 5 (1):10181. doi.org/10.1038/srep10181.

35. Odeh D, Oršolić N, Adrović E, Gaćina L, Perić P, Odeh S, Balta V, Lesar N, Kukolj M. Effects of Volatile Anaesthetics and Iron Dextran on Chronic Inflammation and Antioxidant Defense System in Rats. Antioxidants. 2022; 11.4:708. Doi.org/ 10.3390/antiox11040708.

36. Altahir BM, Al-Awadi AQ, Shakir SM, Abdulazeez O. Biological Assessment, Heamatological Study, and Environmental Detection of Eugenol. International Journal of ChemTech Research. 2020, 13.2:9-17. doi.org/10.20902/IJCTR.2019.130202.

37. Sarhan WS, Fadhil RM, Jawad AS. Effect of Iron Overload on Some Physiological and Biochemical Variables in Immature Female Rats. EXECUTIVE EDITOR. 2019; 10.2:515. doi.org/10.5958/0976-5506.2019.00343.7.

38. Zahran E, Risha E, Rizk A. Comparison propofol and eugenol anesthetics efficacy and effects on general health in Nile Tilapia. Aquaculture. 2021; 534:736251. doi.org/10.1016/j.aquaculture.2020.736251.

39. Barboza JN, da Silva Maia Bezerra Filho C, Silva RO, Medeiros JV, de Sousa DP. An overview on the anti-inflammatory potential and antioxidant profile of eugenol. Oxidative medicine and cellular longevity. 2018; 2018:1-9. doi.org/10.1155/2018/3957262.

40. Patino E, Doty SB, Bhatia D, Meza K, Zhu YS, Rivella S, Choi ME, Akchurin O. Carbonyl iron and iron dextran therapies

cause adverse effects on bone health in juveniles with chronic kidney disease. Kidney international. 2020; 98.5:1210-24. doi.org/ 10.1016/j.kint.2020.05.043.

41. Özbolat G, Yegani AA. Effects of curcumin on iron overload in rats. Sudan Journal of Medical Sciences. 2021; 16.4:464-75. doi.org/10.18502/sjms.v16i4.9944.

42. Chen CJ, Lin TY, Wang CL, Ho CK, Chuang HY, Yu HS. Interactive effects between chronic lead exposure and the homeostatic iron regulator transport HFE polymorphism on the human red blood cell mean corpuscular volume (MCV). International journal of environmental research and public health. 2019; 16.3:354. doi.org/ 10.3390/ijerph16030354.

43. Deng HF, Yue LX, Wang NN, Zhou YQ, Zhou W, Liu X, Ni YH, Huang CS, Qiu LZ, Liu H, Tan HL. Mitochondrial iron overload-mediated inhibition of Nrf2-HO-1/GPX4 assisted ALI-induced nephrotoxicity. Frontiers in Pharmacology. 2021; 11:624529. doi.org/ 10.3389/fphar.2020.624529.

44. Williams GA, Mafimidiwo AN, Olayemi WA, Akinjute OF, Williams OK, Ogunrombi JO. Effect of dietary supplementation with Ethiopian pepper (Xylopia aethiopica), cloves (Syzygium aromaticum), and their composite on growth performance, serum parameters, and haematological indices of broiler chickens. Animal Science and Genetics. 2023; 19.1:1-19. doi.org/10.5604/01.3001.0016.2754.

45. Restrepo-Gallego M, Díaz LE. Influence of dietary vitamin A and iron deficiency on hematologic parameters and body weight of young male wistar rats. Journal of the American Association for Laboratory Animal Science. 2020; 59.1:17-23. doi.org/10.30802/AALAS-JAALAS-19-000020.

46. Dahi AA, Hanafy E, Al Pakra M. Iron overload and platelet function defects: possible correlation. Journal of Investigative Medicine High Impact Case Reports. 2016; 4.4:1-4.doi.org/ 10.1177/2324709616675645.

47. Schrauben SJ, Berns JS. Hematologic Complications of Chronic Kidney Disease—Anemia and Platelet Disorders. 2nd ed; In: Paul LK, Mark ER, Editors.Chronic renal disease, Academic Press: E-Publishing Inc; 2020, p. 463-475. doi.org/10.1016/B978-0-12-815876-0.00030-9.

48. Chaichompoo P, Qillah A, Sirankapracha P, Kaewchuchuen J, Rimthong P, Paiboonsukwong K, Fucharoen S, Svasti S, Worawichawong S. Abnormal red blood cell morphological changes in thalassaemia associated with iron overload and oxidative stress. Journal of Clinical Pathology. 2019:205775. doi.org/10.1136/jclinpath-2019-205775.

49. Faisal HG, Rasheed KN, Marbut MM. The Effect of Iron Overload on Erythrocyte Histological Composition, Insulin-like Growth Factor, and Liver Function in Beta Thalassemia Patients. Journal of Population Therapeutics and Clinical Pharmacology. 2022; 29.02:157-170. doi.org/10.47750/jptcp.2022.958

50. Kumar A, Siddiqi NJ, Alrashood ST, Khan HA, Dubey A, Sharma B. Protective effect of eugenol on hepatic inflammation and oxidative stress induced by cadmium in male rats. Biomedicine & Pharmacotherapy. 2021; 139:111588. doi.org/10.1016/j.biopha.2021.111588.